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Purinoceptors in the rat heart

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1 The effects of an intracoronary bolus of adenosine triphosphate (ATP), α,β-methylene ATP (APCPP), β,γ-methylene ATP (APPCP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine on coronary tone and ventricular myocardial contraction were investigated in the perfused rat heart.

2 Adenine nucleotides, given by bolus injection were negatively inotropic in amounts $> 3 \times 10^{-7}$ mol. The potency order was ATP > ADP > AMP. Adenosine ($< 1 \times 10^{-5}$ mol) had no effect on ventricular myocardial contraction.

3 Adenine nucleotides and adenosine (1×10^{-10}–1×10^{-7} mol) reduced coronary tone. The potency order was ATP > ADP > AMP = adenosine. The ATP analogue APPCP was less active than ATP at reducing coronary tone, and APCPP had no vasodilator effect. This suggests the presence of a P_2-purinoceptor, subclass P_{2Y}, which mediates vasodilatation.

4 ATP and ADP increased the concentration of prostacyclin (measured as 6-keto prostaglandin $F_{1\alpha}$) in the perfusate, but only after injection of $> 3 \times 10^{-7}$ mol, suggesting that the vasodilator responses to ATP and ADP were not mediated by prostacyclin. AMP and adenosine had no effect, even at 1×10^{-5} mol.

5 At a dose of 3×10^{-9} mol, approximately 40% of ATP and 70% of ADP was converted to AMP and adenosine whilst passing through the heart. The amounts of AMP and adenosine formed, however, were insufficient to account for the vasodilator effects of ATP and ADP.

6 Vasodilatation mediated by AMP and adenosine was inhibited by an infusion of 8-phenyltheophylline (8-PT; 2×10^{-5} M) indicating interaction with a P_1-purinoceptor. Vasodilatation induced by ATP (at doses at which AMP and adenosine had no action) was also depressed by 8-PT indicating either an action of ATP on P_1-purinoceptors, or an effect of 8-PT on P_{2Y} receptors.

7 Vasodilatation induced by AMP was unaltered during an infusion of α,β-methylene ADP (2×10^{-6} M, which inhibited breakdown of AMP to adenosine by $54.2 \pm 1.5\%$, $n = 4$). This suggests that AMP acted directly, and it did not require conversion to adenosine to induce vasodilatation.

8 The ATP analogues APCPP (1×10^{-9}–1×10^{-8} mol) and APPCP (1×10^{-8}–1×10^{-7} mol) increased coronary tone, as did high doses (1×10^{-5} mol) of ATP and ADP, indicating the presence of an additional P_2-purinoceptor, subclass P_{2X}, mediating vasoconstriction.

Introduction

The potent cardiovascular actions of extracellular adenine nucleotides and adenosine were first described by Drury & Szent-Gyorgyi (1929) and by Green & Stoner (1950). More recently these agents have been shown to alter myocardial performance (Moir & Downs, 1972; Hopkins, 1973; Collis & Pettinger, 1982; Burnstock & Meghji, 1983) and to reduce coronary resistance (Winbury *et al.*, 1953; Wolf & Berne, 1956; Moir & Downs, 1972; Paddle & Burnstock, 1974). Adenosine is now widely accepted as an agent involved in the regulation of coronary tone (see Berne, 1980 for a review). Adenine nucleotides may be present in the coronary circulation as a result of release from hypoxic myocardium (Paddle & Burnstock, 1974; Forrester & Williams, 1977), from endothelial cells (Pearson & Gordon, 1979) from damaged vessel walls (Born & Kratzer, 1984), and from aggregating platelets (Ingerman *et al.*, 1979), in sufficient amounts to cause local effects on the vasculature and the myocardium.

Adenine nucleotides are rapidly degraded to adenosine as they pass through the coronary circulation (Baer & Drummond, 1968; Hopkins, 1973; Paddle & Burnstock, 1974; Schwartzman *et al.*, 1981; Ronca Testoni & Borghini, 1982) and it is not clear to what extent their cardiac and coronary actions are due to

their intrinsic activity or to the activity of their immediate metabolites. Distinguishing between the effects of adenosine triphosphate (ATP) and adenosine offers the possibility of modulating the effects of released nucleotides by interfering with their metabolism.

This study set out to investigate the effects of adenine nucleotides on cardiac function and coronary tone in the perfused rat heart, to establish what types of receptors were involved, and to determine whether these effects were direct actions of the adenine nucleotides or due to their conversion to adenosine. Additionally, because ATP induces the production of prostaglandin (including prostacyclin) by various vascular beds and by cultured endothelial cells (Minkes *et al.*, 1973; Boeynaems & Galand, 1983; Pearson *et al.*, 1983; Hellewell & Pearson, 1984) we examined whether stimulation of prostaglandin production was involved in mediating the effects of nucleotides on rat coronary vessels.

Methods

Male Sprague-Dawley rats (200–300 g) were anaesthetized by intraperitoneal injection of a mixture of midazolam HCl (0.07 mg kg^{-1}), fentanyl citrate (0.17 mg kg^{-1}) and fluanisone (5.4 mg kg^{-1}). Heparin (500 u) was given intravenously. The hearts were excised, immersed in ice-cold buffer and dissected free of connective tissue. The aorta was cannulated and the coronary circulation perfused by the Langendorff method with a solution containing (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.9, $MgSO_4$ 1.9, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, glucose 5.5, sodium pyruvate 5.5, gassed with O_2:CO_2 (95:5 by volume). Flow, controlled by a roller pump, was increased gradually until perfusion pressure (measured from a side arm of the aortic cannula) reached 60 mmHg (flow = 54.4 ± 1.6 ml min^{-1} g^{-1} dry tissue, mean ± s.e.mean of 65 experiments). Perfusate and tissue temperature were maintained at 37°C. Hearts were electrically stimulated (4 Hz, 12 ms with a supramaximal voltage) by a pair of platinum electrodes inserted into the right ventricle. Myocardial contraction was monitored by a water filled latex balloon within the left ventricle inflated such that left ventrical diastolic pressure did not exceed 10 mmHg. The preparations were allowed to equilibrate for 30 min before addition of drugs. No heart was perfused for longer than 120 min.

ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, α,β-methylene ATP (APCPP) or β,γ-methylene ATP (APPCP) were injected directly into the aortic cannula (50 μl, over 5 s). Changes in perfusion pressure and myocardial contractile force was monitored. Hearts in which ATP or adenosine (3×10^{-8} mol) reduced perfusion pressure by less than 3 mmHg were excluded from the study.

In some experiments the response to a bolus injection of adenine nucleotide was assessed in the presence of a background infusion of one of the following compounds: 8-phenyltheophylline (8-PT, 2×10^{-5} M), α,β-methylene ADP (APCP, 2×10^{-6} M), phentolamine (1×10^{-5} M), atropine (1×10^{-6} M) or methysergide (5×10^{-6} M).

The breakdown of adenine nucleotides in the coronary circulation was measured using 2-^{3}H-adenine nucleotides. 2-[^{3}H]-ATP, -ADP or -AMP (10 μ Ci) was injected into the aortic cannula and the effluent from the heart was collected for 50 s. The proportion of ^{3}H-nucleotides and nucleosides in this effluent was determined by counting on a t.l.c. linear analyser (Berthold LB 284) after t.l.c. separation using the method of Norman *et al.* (1974).

In other experiments the purine composition of the effluent was assessed by h.p.l.c. Adenine nucleotides were analysed using a 250 × 5 mm ODS Hypersil 5 μm column and a mobile phase of 5×10^{-2} M ammonium dihydrogen phosphate at a flow rate of 1 ml min^{-1} (Simmonds *et al.*, 1982). A linear 20 min gradient of 0–30% methanol was used for the analysis of adenosine. Absorbance was measured at 254 nm, and concentrations of nucleotides and nucleosides in samples were determined by quantifying peak areas relative to those produced by known standards.

In selected experiments prostacyclin production was assessed. Perfusate from the heart was collected in 3 s samples for 5 min after adenine nucleotide injection. The prostacyclin concentration in these samples was measured by radioimmunoassay of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) as previously described (Ager *et al.*, 1982), using antiserum generously provided by Dr B.A. Peskar (Bochum, West Germany). The perfusate (0.1 or 0.3 ml) was added to the assay tubes without any solvent extraction. The detection limit of the assay was 3 pg per sample.

Drugs

Adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), α,β-methylene ADP (APCP), α,β-methylene ATP (APCPP), β,γ-methylene ATP (APPCP) and 8-phenyltheophylline (8-PT) were purchased from Sigma, methysergide was obtained from Sandoz. APPCP, APCPP and APCP, were free of contaminating adenine nucleotides and nucleosides (h.p.l.c. analysis).

All drugs except 8-PT were dissolved in distilled water. 8-PT was dissolved in 80% methanol in 2×10^{-1} M NaOH to give a stock solution of 2×10^{-2} M. The vehicle had no effect on perfusion pressure or myocardial contraction.

Results

Effects on ventricular myocardial function

Bolus injections (1×10^{-7} mol) of ATP, ADP or AMP, had no effect on myocardial contraction. Higher doses (3×10^{-7}–1×10^{-5} mol) had transient negative inotropic effects (Figure 1). The minimum dose needed to reduce myocardial contractile force was 3×10^{-7} mol for ATP, 1×10^{-6} mol for ADP and 2×10^{-6} mol for AMP. The highest dose of ATP, ADP or AMP used (1×10^{-5} mol) reduced myocardial contractile force by 96.2 ± 2.4% ($n = 5$), 84.4 ± 3.7 ($n = 5$) and 76.3 ± 4.4% ($n = 5$) respectively (all results are expressed as mean ± s.e.mean of n experiments). Adenosine (up to 1×10^{-5} mol) showed no negative inotropic activity (Figure 1).

Effects on coronary perfusion pressure

Adenine nucleotides and adenosine had effects on perfusion pressure at doses lower than those required to produce inotropic effects. Low doses (3×10^{-10}–1×10^{-7} mol) of ATP, ADP, AMP and adenosine transiently reduced perfusion pressure (Figure 2). This decrease peaked after 16 ± 1 s ($n = 30$) and returned to control values within 1 min (Figure 3). ATP and ADP were approximately equiactive and about ten times more potent than adenosine (Figure 2): maximum reduction in perfusion pressure occurred after injection of 1×10^{-8} mol ATP or ADP and 1×10^{-7} mol adenosine. With high doses of ATP and ADP (1×10^{-5} mol), which were negatively inotropic, an increase in perfusion pressure (6.5 ± 1.5 and 3.9 ± 1.2 mmHg respectively, $n = 5$) was seen, which peaked after 30 ± 2 s and returned to control values within 2 min (Figure 3).

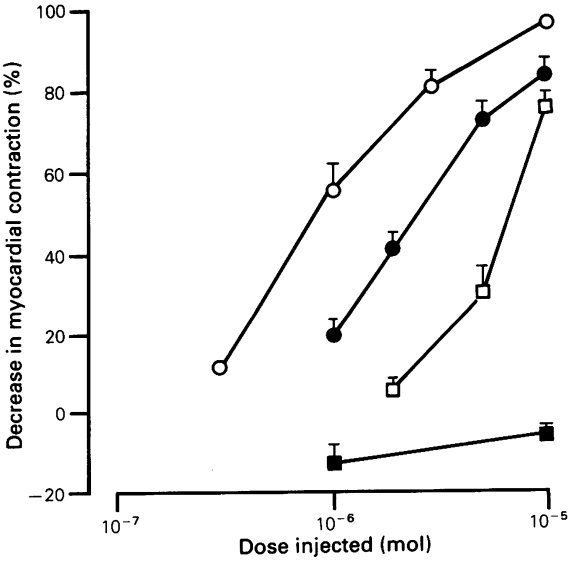

Figure 1 Effect of a 50 μl bolus of ATP (○), ADP (●), AMP (□) and adenosine (■), on ventricular myocardial contractile force. Vertical lines show s.e.mean (when larger than symbol) of 5 experiments.

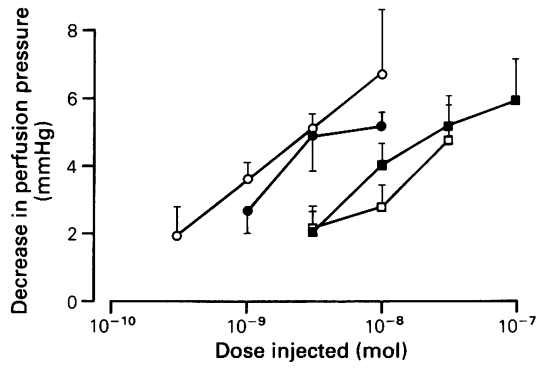

Figure 2 Effect of a 50 μl bolus of ATP (○), ADP (●), AMP (□) and adenosine (■) on perfusion pressure. Vertical lines show s.e.mean of 5 or more experiments.

Analogues of ATP also altered perfusion pressure (Figures 3 and 4); APPCP initially increased perfusion pressure in a dose-related manner (1×10^{-8}–1×10^{-7} mol) but this was rapidly followed by a reduction in perfusion pressure with a similar time course and over a similar dose range to adenosine. APCPP was about ten times more potent that APPCP at increasing perfusion pressure (Figure 4): APCPP (1×10^{-8} mol) increased perfusion pressure by 11.6 ± 3.9 mmHg ($n = 5$) whilst the same dose of APPCP increased it by only 3.6 ± 0.9 mmHg ($n = 7$). The response to APCPP peaked after 9 ± 1 s and perfusion pressure returned to control values within 2 min, there was no subsequent reduction in perfusion pressure (Figures 3 and 4). The response to APCPP was unaltered in the presence of a background infusion of atropine (1×10^{-6} M), methysergide (5×10^{-6} M) or phentolamine (1×10^{-5} M) $n = 3$, indicating that the response was not mediated by release of acetylcholine, 5-hydroxytryptamine, or noradrenaline.

When 2-^{3}H-adenine nucleotides were injected into the coronary circulation over 99% of the tritium recovered in the perfusate was collected within 39.5 ± 0.4 s ($n = 8$) – i.e. in a volume of 8.09 ± 0.21 ml ($n = 8$). Sequential collection of 1 s samples of effluent showed that a maximum of 10.8 ± 0.1% ($n = 8$) of the recovered tritium was collected in any sample. Thus

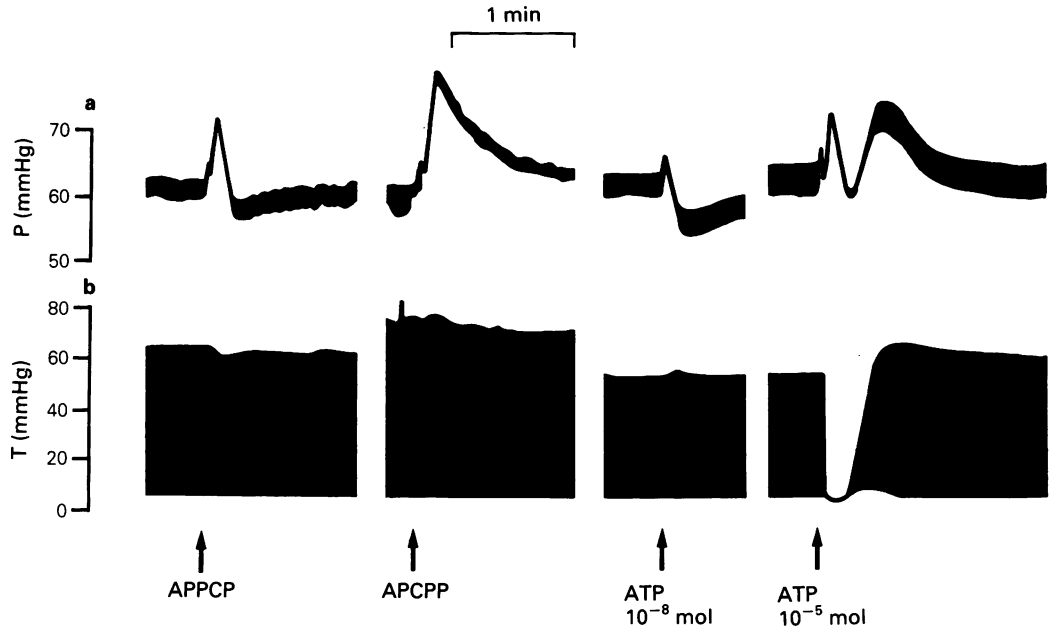

Figure 3 Experimental records of the effect of perfusion pressure (a) and myocardial contractile function (b) of: β,γ-methylene ATP (APPCP, 1×10^{-7} mol); α,β-methylene ATP (APCPP, 1×10^{-8} mol) and ATP, 1×10^{-8} mol and 1×10^{-5} mol. Note initial small increase in perfusion pressure due to the force of injection (at arrow) and the decrease in perfusion pressure caused by the negative inotropic action of the high dose of ATP (far right).

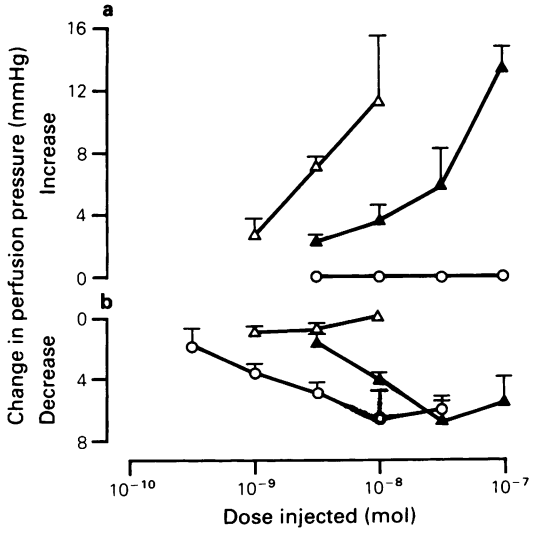

Figure 4 Comparison of the effect on perfusion pressure of ATP (○) with that of α,β-methylene ATP (△) and β, γ-methylene ATP (▲). (a) Initial increase in perfusion pressure, (b) the subsequent decrease in perfusion pressure. Vertical lines show s.e.mean of 5 or more experiments.

the mean concentration within the coronary circulation was calculated (in nmol ml^{-1}) as:

$$\frac{\text{dose injected (nmol)}}{\text{volume of distribution (ml)}} = \text{dose injected} \times 0.124,$$

and the peak concentration reached (in nmol ml^{-1}) as:

$$\frac{\text{dose injected (nmol)} \times \text{max. recovered in any fraction} \times 60}{\text{flow rate (ml min}^{-1})} = \text{dose injected} \times 0.536$$

Thin layer chromatography of the perfusate showed that 2-^{3}H-adenine nucleotides were metabolized as they passed through the coronary circulation (Table 1). When a dose of 3×10^{-9} mol of ATP or ADP was injected 37 ± 7% ($n = 6$) of the former and 69 ± 6% ($n = 5$) of the latter was converted to AMP or adenosine. The amounts of AMP and adenosine formed ($1.11 \pm 0.20 \times 10^{-9}$ mol and $2.08 \pm 0.18 \times 10^{-9}$ mol, respectively) were lower than those required to reduce perfusion pressure when these agents were injected directly into the coronary circulation, they were therefore insufficient to account for the decrease in perfusion pressure observed.

Assessment of the breakdown of APPCP and

Table 1 Metabolism of adenine nucleotides in the coronary circulation

		% 2-^{3}H-nucleotide on t.l.c. plate collected as					
	Dose injected	*ATP*	*ADP*	*AMP*	*Adenosine*	*Inosine*	n
ATP	3×10^{-9} mol	48 ± 7	11 ± 1	25 ± 4	12 ± 3	4 ± 1	6
	3×10^{-7} mol	54 ± 6	10 ± 1	21 ± 3	11 ± 3	3 ± 1	6
ADP	3×10^{-9} mol	—	19 ± 4	43 ± 3	26 ± 4	11 ± 5	5
	3×10^{-7} mol	—	22 ± 5	36 ± 7	29 ± 10	13 ± 4	5
AMP	3×10^{-8} mol	—	—	41 ± 8	54 ± 7	5 ± 3	4
	3 ± 10^{-7} mol	—	—	50 ± 12	50 ± 11	1 ± 1	3

Results show mean ± s.e.mean of *n* observations.

APCPP by h.p.l.c. showed that 78 ± 3% ($n = 4$) of a 3×10^{-8} mol dose of APPCP and 88 ± 5% ($n = 4$) of the same dose of APCPP remained as the parent compound after passage through the coronary bed.

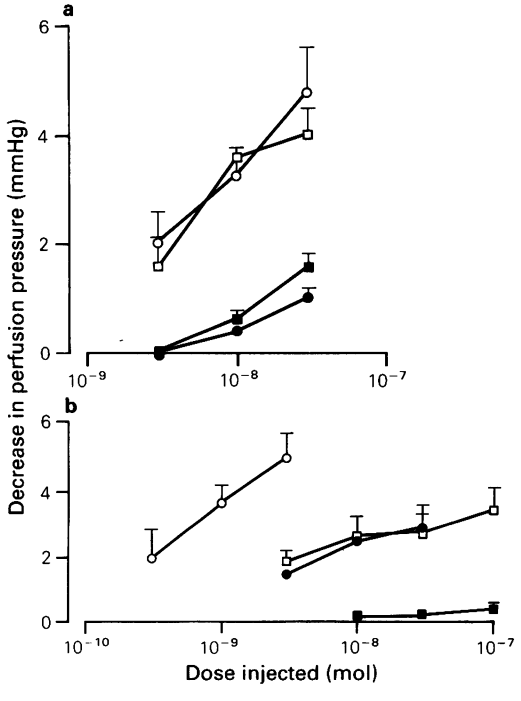

Figure 5 Effect of 8-phenyltheophylline (8-PT, 2×10^{-5} M) on the decrease in perfusion pressure in response to (a) a 50 μl bolus of AMP ((○) before, (●) after 8-PT) or adenosine ((□) before, (■) after 8-PT) and (b) a 50 μl bolus of ATP ((○) before, (●) after 8-PT) or β,γ-methylene ATP ((□) before, (■) after 8-PT). Vertical lines show s.e.mean of 5 experiments.

Inhibition of the formation or action of adenosine

8-Phenyltheophylline (8-PT) is known to inhibit competitively the interaction of adenosine with P_1-purinoceptors (Griffith *et al.*, 1981). In our experiments the vehicle in which 8-PT was dissolved had no effect on the vascular responses to adenine nucleotides or adenosine. A continuous infusion of 8-PT (2×10^{-5} M) abolished the response to 3×10^{-9} mol AMP and adenosine and severely depressed the responses induced by 1 and 3×10^{-8} mol (Figure 5a). The response to ATP (3×10^{-9}–3×10^{-8} mol) was also greatly reduced (Figure 5b), and the decrease in perfusion pressure caused by APPCP (1×10^{-8}–1×10^{-7} mol) was virtually abolished (Figure 5b).

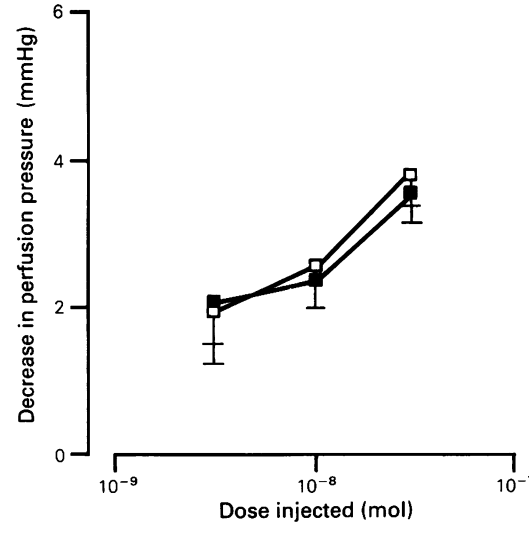

Figure 6 Effect of α,β-methylene ADP (APCP, 2×10^{-6} M) on the decrease in perfusion pressure in response to a 50 μl bolus of AMP ((□) before, (■) after APCP). Vertical lines show s.e.mean of 5 experiments.

APCP, a potent inhibitor of 5'-nucleotidase (Naito & Lowenstein, 1985), had no intrinsic vasoactivity in our experiments (up to 1×10^{-7} mol). A continuous infusion of 2×10^{-6} M had no significant effect on the reduction in perfusion pressure caused by AMP (Figure 6). H.p.l.c. analysis showed that in the absence of APCP $22.3 \pm 3.4\%$ ($n = 4$) of a 3×10^{-8} mol dose of AMP remained as the parent compound whilst $46.0 \pm 6.7\%$ was converted to adenosine. In the presence of APCP (2×10^{-6} M) the corresponding figures were $76.4 \pm 4.5\%$ AMP and $12.5 \pm 1.4\%$ adenosine ($n = 4$). Thus, the effect of AMP did not depend on its conversion to adenosine.

Prostaglandin release

Under basal conditions prostacyclin (measured as 6-keto $PGF_{1\alpha}$) was released into the perfusate from the heart (0.69 ± 0.04 ng min^{-1}; $n = 15$). ATP (3×10^{-7}–1×10^{-5} mol) induced a dose-related stimulation of prostacyclin release (Figure 7). The absolute amount of prostacyclin released into the effluent varied widely between hearts (the peak response to 3×10^{-6} mol ATP ranged from 0.72 ng min^{-1} to 22.33 ng min^{-1}), resulting in mean values with large standard errors.

Prostacyclin released in response to ATP peaked within 40 s of injection and returned to baseline values within 5 min. ADP stimulated prostacyclin release with a similar time course to ATP but was slightly less potent (mean maximal stimulation to 1×10^{-5} mol of ADP was 8.29 ± 2.90 ng min^{-1}, compared with 11.93 ± 3.23 ng min^{-1} in response to the same dose of ATP). AMP, adenosine (both up to 1×10^{-5} mol), APPCP and APCPP (both up to 1×10^{-7} mol) did not stimulate prostacyclin production.

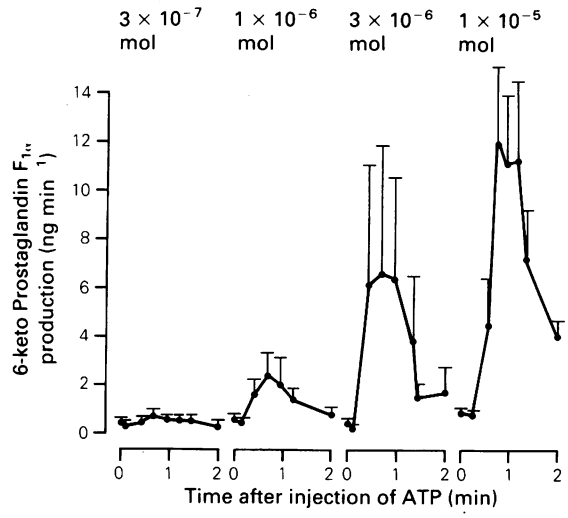

Figure 7 Stimulation of prostacyclin production (measured as 6-keto prostaglandin $F_{1\alpha}$) in response to ATP (3×10^{-7}–1×10^{-5} mol). Vertical lines show s.e.mean of 4 or more experiments.

Discussion

Adenine nucleotides are most likely to occur extracellularly within the coronary circulation as a result of platelet degranulation or release from damaged cells in the blood vessel wall. Because such release will be transient and localized, we studied the effects of bolus injections, rather than continuous infusion, on ventricular myocardial function and coronary tone.

Effects on myocardial function

Adenine nucleotides and adenosine have been demonstrated to be negatively inotropic in atrial myocardium (Drury & Szent-Gyorgyi, 1929; Collis & Pettinger, 1982; Burnstock & Meghji, 1983) and to slow sinoatrial conduction (James, 1965). The majority of studies on ventricular myocardial function show little or no effect of adenine nucleotides on ventricular conduction rate or force of contraction, although Burnstock & Meghji (1983) showed negative inotropism with high concentrations (3×10^{-4}–1×10^{-3} M) of ATP in the right ventricular strip of the rat, and a decrease in ventricular pacemaker rate has been found in guinea-pig isolated heart in the presence of adenosine (Szentmiklosi *et al.*, 1980; West *et al.*, 1982). In our experiments where chronotropic effects were prevented by electrical pacing and only ventricular function was measured, relatively high doses of adenine nucleotides ($> 3 \times 10^{-7}$ mol) were needed to produce negative inotropism. The intracoronary concentrations reached under these conditions; mean concentrations 3.7×10^{-5} M, peak concentration 1.6×10^{-4} M (estimated from the distribution profile of injected [^{3}H]-ATP), were similar to those used by Burnstock & Meghji (1983). The relative potencies of ATP, ADP and AMP, and the absence of a response to adenosine, indicate that the negative inotropic response is mediated by a P_2-purinoceptor (as defined by Burnstock, 1978).

Effects on perfusion pressure

In the perfused heart of the rat, where coronary flow is kept constant, any change in perfusion pressure, in the absence of inotropic effects on the myocardium reflects an alteration in coronary smooth muscle tone. In our experiments ATP, ADP, AMP and adenosine reduced coronary tone, APCPP increased it, and

APPCP and high doses of ATP and ADP had a biphasic effect.

Previous studies have shown that adenosine produces vasodilatation in many vascular beds, including the coronary circulation (see Berne, 1980, for a review) through stimulation of P_1-purinoceptors (as defined by Burnstock, 1978). Adenine nucleotides initiate endothelium-dependent relaxation of arterial smooth muscle (i.e. vasodilatation) in various vessels by interaction with endothelial P_2-purinoceptors (De Mey & Vanhoutte, 1981; Rapaport *et al.*, 1984; Kennedy *et al.*, 1985; White *et al.*, 1985). Stimulation of P_2-purinoceptors on smooth muscle cells in some vessels has been found to increase vascular tone (Verhaeghe, 1977; Su, 1981; Kennedy *et al.*, 1985; Kennedy & Burnstock, 1985a; White *et al.*, 1985).

Our experiments were designed to establish the type of purinoceptor (P_1 or P_2) responsible for the observed effects in the rat coronary circulation. The relative potencies of ATP and adenosine in altering coronary tone suggests that the receptor type primarily involved in the vasodilator response is P_2, although vasodilatation to adenosine may be mediated by interaction with a P_1-purinoceptor. Inhibiting 5'-nucleotidase with APCP had no effect on the response to AMP, suggesting that conversion to adenosine was not necessary for a vasodilator action and that AMP was either equipotent with adenosine at the P_1-purinoceptor or was a less potent agonist than ATP and ADP at the P_2-purinoceptor. The vasodilatation in response to adenosine was inhibited by 8-PT, a competitive antagonist at P_1-purinoceptors (Griffith *et al.*, 1981). However, 8-PT also inhibited the vasodilatation in response to ATP at doses where the amount of adenosine formed was below the response threshold. Thus, either ATP interacts with P_1-purinoceptors or, more probably, 8-PT is an antagonist at the vasodilator P_2-receptor.

Burnstock & Kennedy (1985) recently suggested that P_2-purinoceptors should be subdivided into two types on the basis of the relative potencies of ATP analogues and ATP. According to this classification, the decrease in coronary tone seen in our experiments is mediated by a P_{2Y}-purinoceptor (ATP more potent than APCPP and APPCP) as found in rabbit portal vein (Kennedy & Burnstock, 1985b), rat femoral artery (Kennedy *et al.*, 1985) and rat aorta (White *et al.*, 1985). The increase that we observed in coronary tone (with APCPP and APPCP more potent than ATP) is mediated by a P_{2X}-purinoceptor, as found in rat femoral artery (Kennedy *et al.*, 1985), rabbit ear artery (Kennedy & Burnstock, 1985a) and rat aorta (White *et al.*, 1985).

Interaction with P_2-purinoceptors on endothelial cells can induce increased prostacyclin production (Boeynaems & Galand, 1983; Pearson *et al.*, 1983) and prostacyclin is a potent vasodilator in many vascular beds including the coronary circulation of the rat (Schror *et al.*, 1980). In our experiments doses of ATP able to induce vasodilatation were one thousand times lower than those required to increase the prostacyclin concentration in the perfusate. Thus the vasodilatation produced by stimulation of P_{2Y}-receptors in this system was not mediated by prostacyclin.

In some smooth muscle preparations, increases in tone induced by ATP analogues have been shown to be mediated through release of acetylcholine (Moody & Burnstock, 1982), 5-hydroxytryptamine (Sakai, 1978) or noradrenaline (Su, 1981) from nerve endings. However, in our experiments atropine, methysergide and phentolamine had no effect on the response to APCPP, indicating that these neurotransmitters were not involved in purinoceptor-mediated vasoconstriction in the rat heart.

Conclusions

The rat coronary vasculature exhibits purinoceptors of at least two types: a vasodilator P_2-purinoceptor (P_{2Y}) stimulated by low concentrations of ATP and ADP, and a P_2-purinoceptor mediating vasoconstriction (P_{2X}), stimulated by higher concentrations of ATP and ADP and by slowly degraded ATP analogues. AMP and adenosine may act as less potent agonists at the P_2-purinoceptor or their effects may indicate the presence of an additional vasodilator P_1-purinoceptor. High concentrations of ATP and ADP also stimulate prostacyclin release and are negatively inotropic.

Significant extracellular concentrations of adenine nucleotides occurring locally within the coronary vasculature will normally increase coronary flow by stimulating vasodilator purinoceptors, thus limiting the extent of platelet aggregation and relieving hypoxia. If, as in the rat femoral artery (see Kennedy *et al.*, 1985), the vasoconstrictor P_{2X}-receptors are on smooth muscle cells whilst those mediating vasodilatation (P_{2Y}) are on endothelial cells then damage to the endothelial cells may result in a shifting of the ATP-mediated response from one of dilatation to one of constriction, especially if the metabolism of ATP and ADP is impaired because endothelial ectonucleotidase activity is compromised. Under such circumstances, increases in extracellular ATP and ADP could lead to further platelet aggregation, isolation of the hypoxic area by vasoconstriction and eventual myocardial infarction.

References

AGER, A., GORDON, M.L., MONCADA, S., PEARSON, J.D., SALMON, J.A. & TREVETHICK, M.A. (1982). Effects of isolation and culture on prostaglandin synthesis by porcine aortic endothelial and smooth muscle cells. *J. Cell Physiol.*, **110**, 9–16.

BAER, H.P. & DRUMMOND, G.I. (1968). Catabolism of adenine nucleotides by the isolated perfused rat heart. *Proc. Soc. exp. Biol. Med.*, **127**, 33–36.

BERNE, R.M. (1980). The role of adenosine in the regulation of coronary blood flow. *Circulation Res.*, **47**, 807–813.

BOEYNAEMS, J.M. & GALAND, N. (1983). Stimulation of vascular prostacyclin synthesis by extracellular ADP and ATP. *Biochem. biophys. Res. Commun.*, **112**, 290–295.

BORN, G.V. & KRATZER, M.A. (1984). Source and concentration of extracellular adenosine triphosphate during haemostasis in rats, rabbits and man. *J. Physiol.*, **354**, 419–429.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*, ed. Bolis, L. & Straub, R.B, pp. 107–118, New York: Raven Press.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmac.*, **16**, 433–440.

BURNSTOCK, G. & MEGHJI, P. (1983). The effect of adenyl compounds on the rat heart. *Br. J. Pharmac.*, **79**, 211–218.

COLLIS, M.G. & PETTINGER, S.J. (1982). Can ATP stimulate P_1-receptors in guinea-pig atrium without conversion to adenosine. *Eur. J. Pharmac.*, **81**, 521–529.

DE MEY, J.G. & VANHOUTTE, P.M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.*, **316**, 347–355.

DRURY, A.N. & SZENT-GYORGYI, A. (1929). The physiological activity of the adenine compounds with reference to action upon the mammalian heart. *J. Physiol.*, **68**, 213–237.

FORRESTER, T. & WILLIAMS, C.A. (1977). Release of adenosine triphosphate from isolated adult heart cells in response to hypoxia. *J. Physiol.*, **268**, 371–390.

GREEN, H.N. & STONER, H.B. (1950). *Biological Actions of the Adenine Nucleotides*. London: H.K. Lewis & Co Ltd.

GRIFFITH, S.G., MEGHJI, P., MOODY, C.J. & BURNSTOCK, G. (1981). 8-Phenyltheophylline: a potent P_1-purinoceptor antagonist. *Eur. J. Pharmac.*, **75**, 61–64.

HELLEWELL, P.G. & PEARSON, J.D. (1984). Purinoceptor mediated stimulation of prostacyclin release in the porcine pulmonary vasculature. *Br. J. Pharmac.*, **83**, 457–462.

HOPKINS, S.V. (1973). The action of ATP in the guinea-pig heart. *Biochem. Pharmac.*, **22**, 335–339.

INGERMAN, C.M., SMITH, J.B. & SILVER, M.J. (1979). Direct measurement of platelet secretion in whole blood. *Thromb. Res.*, **16**, 335–344.

JAMES, T.N. (1965). The chronotropic action of ATP and related compounds studied by direct perfusion of the sinus node. *J. Pharmac. exp. Ther.*, **149**, 233–247.

KENNEDY, C. & BURNSTOCK, G. (1985a). ATP produces vasodilation via P_1-purinoceptors and vasoconstriction via P_2-purinoceptors in the isolated rabbit central ear artery. *Blood Vessels*, **22**, 145–155.

KENNEDY, C. & BURNSTOCK, G. (1985b). Evidence for two types of P_2-purinoceptor in the longitudinal muscle of the rabbit portal vein. *Eur. J. Pharmac.*, **111**, 49–56.

KENNEDY, C., DELBRO, D. & BURNSTOCK, G. (1985). P_2-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. *Eur. J. Pharmac.*, **107**(2), 161–168.

MINKES. M., DOUGLAS, J.R. & NEEDLEMAN, P. (1973). Prostaglandin release by the isolated perfused rabbit heart. *Prostaglandins*, **3**, 439–445.

MOIR, T.W. & DOWNS, T.D. (1972). Myocardial reactive hyperaemia: comparative effects of adenosine, ATP, ADP and AMP. *Am. J. Physiol.*, **222**, 1386–1390.

MOODY, C.J. & BURNSTOCK, G. (1982). Evidence for the presence of P_1-purinoceptors on cholinergic nerve terminals in the guinea-pig ileum. *Eur. J. Pharmac.*, **77**, 1–9.

NAITO, Y. & LOWENSTEIN, J.M. (1985). 5′-Nucleotidase from rat heart membranes inhibition by adenine nucleotides and related compounds. *Biochem. J.*, **226**, 645–651.

NORMAN, G.A., FOLLETT, M.J. & HECTOR, D.A. (1974). Quantitative thin-layer chromatography of ATP and the products of its degradation in meat tissue. *J. Chromatog.*, **90**, 105–111.

PADDLE, B.M. & BURNSTOCK, G. (1974). Release of ATP from perfused heart during coronary vasodilatation. *Blood Vessels*, **11**, 110–119.

PEARSON, J.D. & GORDON, J.L. (1979). Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. *Nature*, **281**, 384–386.

PEARSON, J.D., SLAKEY, L.L. & GORDON, J.L. (1983). Stimulation of prostaglandin production through purinoceptors on cultured porcine endothelial cells. *Biochem. J.*, **214**, 273–276.

RAPOPORT, R.M., DRAZNIN, M.B. & MURAD, F. (1984). Mechanisms of adenosine triphosphate, thrombin- and trypsin- induced relaxation of rat thoracic aorta. *Circulation Res.*, **55**, 468–479.

RONCA-TESTONI, S. & BORGHINI, F. (1982). Degradation of perfused adenine compounds up to uric acid in isolated rat heart. *J. Mol. Cell. Cardiol.*, **14**, 177–180.

SAKAI, K. (1978). Tryptaminergic mechanism participating in induction of vasoconstriction by adenine nucleotides, adenosine, IMP and inosine in the isolated and blood perfused hind limb preparation of the rat. *Jap. J. Pharmac.*, **28**, 579–587.

SCHROR, K., LINK, H.B., ROSEN, R., KLAUS, W. & ROSEN, P. (1980). Prostacyclin-induced coronary vasodilation. Interactions with adenosine, cyclic AMP and energy charge in the rat heart *in vitro*. *Eur. J. Pharmac.*, **64**, 341–348.

SCHWARTZMAN, M., PINKAS, R. & RAZ, A. (1981). Evidence for different purinergic receptors for ATP and ADP in rabbit kidney and heart. *Eur. J. Pharmac.*, **74**, 167–173.

SIMMONDS, R.J., COADE, S.B., HARKNESS, R.A., DRURY, L. & HYTTEN, F.E. (1982). Nucleotide, nucleoside and purine base concentrations in human placentae. *Placenta*, **3**, 29–38.

SU, C. (1981). Purinergic receptors in blood vessels. In

Purinergic Receptors (Receptors & Recognition Series B), Vol. 12, ed. Burnstock, G. pp. 95–117, London: Chapman & Hall.

SZENTMIKLOSI, A.J., NEMETH, M., SZEGI, J., PAPP, J.G.Y. & SZEKERES, L. (1980). Effect of adenosine in sinoatrial and ventricular automaticity of the guinea-pig. *Naunyn-Schmiedebergs Arch. Pharmac.*, **311,** 147–149.

VERHAEGHE, R.H. (1977). Action of adenosine and adenine nucleotides on dog's isolated veins. *Am. J. Physiol.*, **233,** H 114–121.

WEST, A., BELARDINELLI, L. & BERNE, R.M. (1982). Antagonistic effects of adenosine and aminophylline on ventricular automaticity. *Fedn. Proc.*, **41,** 1384.

WHITE, T.D., CHAUDRY, A., VOHRA, M.M., WEBB, D. & LESLIE, R.A. (1985). Characteristics of P_2 (nucleotide) receptors mediating contraction and relaxation of rat aortic strips: possible physiological relevance. *Eur. J. Pharmac.*, **118,** 37–44.

WINBURY, M.M., PAPIERSKI, D.H., HEMMER, M.L. & HAMBOURGER, W.E. (1953). Coronary dilator action of the adenine-ATP series. *J. Pharmac. exp. Ther.*, **109,** 255–260.

WOLF, M.M. & BERNE, R.M. (1956). Coronary vasodilator properties of purine and pyrimidine derivatives. *Circulation Res.*, **14,** 343–348.

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